THE USE OF A TEMPERATURE-GRADIENT INCUBATOR TO INVESTIGATE THE TEMPERATURE CHARACTERISTICS OF SOME BACTERIA FROM ANTARCTIC PEAT

By J. H. BAKER*

ABSTRACT. The design, construction and use of a temperature-gradient block is described. The block is capable of incubating two separate organisms at 20 different temperatures each with continuous aeration. Five named psychrophilic bacteria previously isolated from Signy Island peat were grown in the block which enabled their cardinal temperatures and temperature characteristics to be defined. The significance of the temperature characteristic (slope of the linear part of an Arrhenius plot) is discussed.

THE majority of aerobic heterotrophic bacteria occurring in Antarctic peat on Signy Island, South Orkney Islands, are known to be psychrophilic (Baker and Smith, 1972). The term psychrophilic is used in the sense of Ingraham and Stokes (1959) to describe those bacteria capable of forming colonies on solid media easily visible to the naked eye within 2 weeks at 0° C. Mesophiles differ from psychrophiles in their minimum growth temperatures as the former are generally unable to reproduce at temperatures below approximately 7° C. At a depth of only 2·5 cm. below the uppermost leaves of a peat-forming moss (*Polytrichum alpestre*) on Signy Island the temperature rarely rises above 5° C (Longton, 1972). Hence it seems probable that for most of the time bacterial activity in Antarctic peat is restricted to the psychrophiles. This paper is concerned with the effect of temperature in laboratory cultures on the growth rates of five psychrophilic bacteria compared with the growth of a mesophilic bacterium, *Salmonella typhimurium*. The five psychrophiles all came from peat formed under *Chorisodontium aciphyllum* moss at a site on the west coast of Signy Island. They were isolated on Oxoid tryptone soya petri plates incubated at 10° C during the course of a programme aimed at enumerating the bacteria from three depths in the peat (Baker, 1970). From a knowledge of the growth rates of the psychrophilic bacteria at many different temperatures, the cardinal points, i.e. minimum, optimum and maximum growth temperatures, may be ascertained. It is recognized that bacterial growth rates obtained in the laboratory differ from those occurring in the field, but laboratory studies are necessary to demonstrate the potentialities of the organisms.

TEMPERATURE-GRADIENT BLOCK

Construction

In order to determine cardinal temperatures accurately, it is necessary to grow the bacterium at many constant temperatures that differ only slightly, e.g. 1° C, from each other. The temperature-gradient block is a convenient, elegant and relatively inexpensive tool for this purpose. There are many different types of temperature-gradient incubator (Dimmick, 1965; Okami and Sasaki, 1967; Packer and others, 1973), but they all work on the simple principle that a regular temperature gradient exists in a material two parts of which are kept at different constant temperatures. Hence, if one end of a metal bar is maintained at 40° C and the ambient temperature remains constant at 20° C, then cultures placed in holes at different positions along the bar will be on a temperature gradient somewhere between 20° and 40° C. For the measurement of maximum growth rates it is necessary to use broth cultures and, if temperature rather than oxygen is to be the limiting parameter, the broth cultures must be adequately aerated. The system employed by Dimmick (1965) of removing each liquid culture every hour and shaking it is laborious and probably inadequate for a fast-growing culture. A temperature-gradient incubator was built for the present study (Fig. 1) in which broth cultures were individually aerated by bubbling sterile air through them.

The basis of the gradient incubator was an aluminium block 61 cm. by 10 cm. by 10 cm., into which was drilled a series of 60 holes in three rows of 20. The distance between the centres

* Present address: The Freshwater Biological Association, East Stoke, Wareham, Dorset.

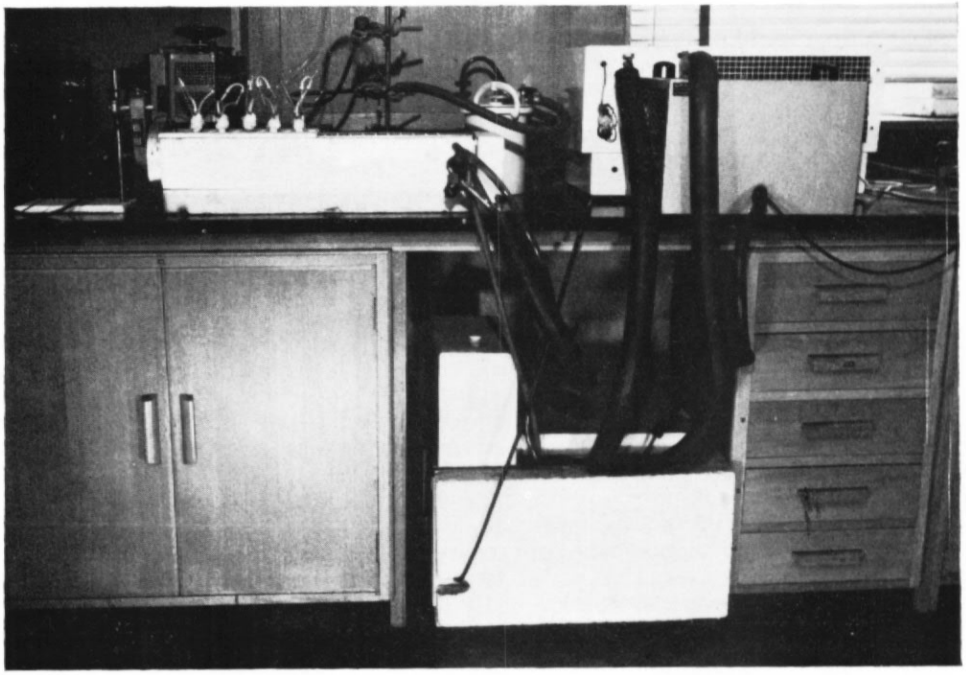

Fig. 1. General view of the temperature-gradient block and associated apparatus. The water bath can be seen on the floor underneath the bench and the cooling units are on the right-hand side of the picture one in front of the other.

of adjacent holes, within and between rows, was 2·54 cm. and the holes were just wide enough to take EEL (Evans Electroselenium Limited) colorimeter tubes which were used as the growth vessels. Part of the block with some of the holes can be seen in Fig. 2. The holes were drilled to a depth of 6·3 cm. so that when the EEL tubes were loaded with broth the centre of the broth was in the centre of the block. The triple row of holes made it possible to incubate two different organisms at the same time at each of 20 separate temperatures. The third row of tubes was used for controlling the temperature of the air supply as described below. A small quantity of mercury was placed in the bottom of each hole to ensure a good thermal contact between the block and the tube. The block was heated at one end by having a hot-plate smeared with electrode jelly pressed firmly on to it. At the other end, six horizontal holes in two staggered lines were drilled right through the width of the block. On one side these holes were joined in pairs by three glass U-tubes; on the other side of the block the holes provided three separate inputs and three exits for cooling solution which was circulated from an LTE (Laboratory Thermal Equipment Limited) water bath at approximately 18 l. min.$^{-1}$. The continuous circulation of the coolant negated the need for a separate stirrer in the bath. The cooling liquid was a 25 per cent aqueous anti-freeze solution which was refrigerated by two independent cooling units. One of the cooling units was a Grant CC 15 continuously operating flat coil fully immersed in the water bath; the other was a LEEC (Laboratory Electrical Engineering Company Limited) thermostatically controlled refrigeration unit originally designed to work in conjunction with a LEEC "Precision" incubator. The LEEC unit pumped a coolant round an LTE triple coil which fitted inside the water bath.

The aluminium block and the LTE bath were insulated with expanded polystyrene 5·1 cm. thick except for the top surface of the block which was insulated with ceiling tiles of a similar material cut to fit. Holes were made in the ceiling tiles with a cork borer to coincide with the holes in the aluminium block. The tubing connections from the cooling units to the water bath and from the water bath to the block were surrounded by neoprene foam insulation.

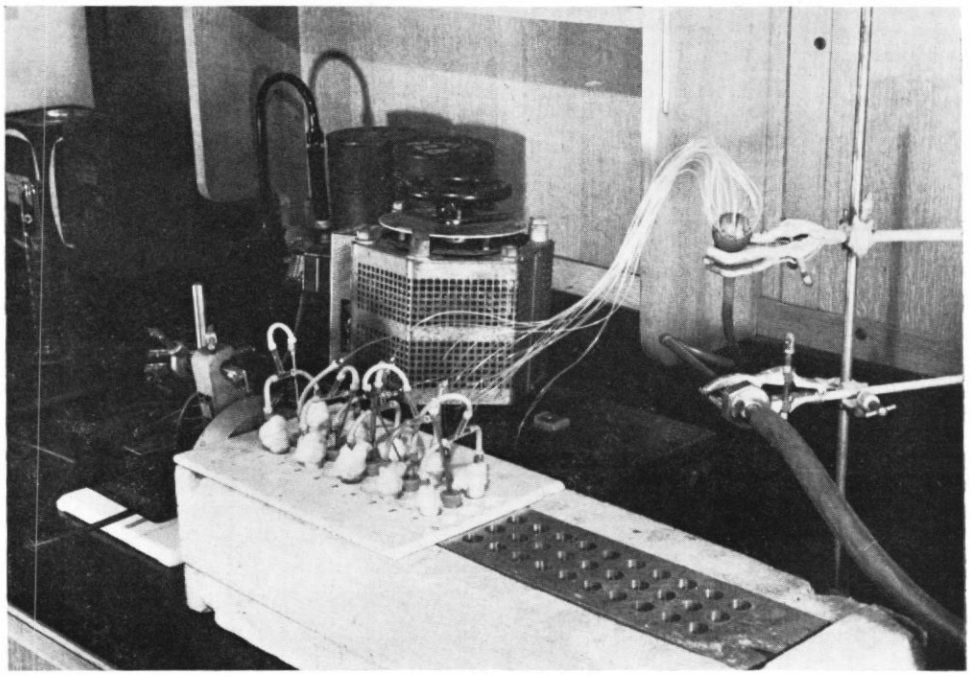

Fig. 2. Temperature-gradient block with the air supply displayed and part of the insulation removed.

The hot-plate was initially controlled by a "Variac" continuously variable transformer and the setting of the "Variac" was determined empirically. It was found that, if the apparatus was switched on and allowed to equilibrate overnight, provided that the ambient temperature remained constant the temperature fluctuation in any tube measured with a mercury-in-glass thermometer was not more than $\pm 0{\cdot}05^{\circ}$ C. Unfortunately it was not always possible to maintain a constant room temperature so further controls had to be used. Thus, if the room temperature fell, the temperature of the cooling liquid was prevented from falling concomitantly by employing the thermostatically controlled heater in the LTE bath. The heater was separated from the Grant coil by a spacer. If the room temperature rose, the block tended to get hotter but this was prevented by automatically shutting off the hot-plate. Regulation of the hot-plate was effected by a thermo-couple (inserted into the block) which activated a "Sunvic" thermostat connected to the "Variac". This discontinuous power supply has the disadvantage that, although large fluctuations are avoided when the room temperature varies, the heating (or cooling) is pulsed, creating waves of heat within the block. In the present apparatus the waves have been minimized at the hot end of the block by placing the thermo-couple as close to the hot-plate as was technically possible. Hence the switching response was as sensitive as possible and the natural damping effect of that part of the block between the thermo-couple and the EEL tubes was maximized. By heating the entire end of the block rather than inserting a heating element as Dimmick (1965) did there was no detectable variation in temperature across the width of the block.

As stated previously, aeration and mixing of the broth in the EEL tubes was effected by bubbling air through the cultures. The air came from the bench supply and was first passed through a ballast chamber consisting of a modified McIntosh and Fildes anaerobic jar. The anaerobic jar was half-filled with distilled water and a sintered glass gas-distribution tube (porosity 1) was attached to the inlet. By passing the air through the water, it was humidified which prevented the excessive evaporation from the broth that occurred when dry air was bubbled through it. The humidified air issuing from the ballast chamber was sterilized by

passing it through a 0·22 μm. membrane filter contained in a Millipore gas-line filter holder. The sterile air was then divided into 20 separate supplies by means of a sterilizable teat manifold designed and produced by the Department of Chemical Physics, University of Surrey. This manifold, which can be seen in Fig. 2, provided an equal pressure on each exit tube, and each exit tube was connected by a close push-fit to a 20 gauge unsharpened 16 mm. hypodermic needle. The luer sockets of the hypodermic needles were connected by non-toxic rubber tubing to 13 gauge stainless steel canular needles which were held by rubber closures in EEL tubes in the centre row of holes in the block. These centre tubes also contained distilled water but this time the water was at the temperature of that part of the block. Thus the air which issued from the centre EEL tubes via a second canular needle was not only humidified but also at the same temperature as the adjacent culture tubes through which it was then bubbled.

Operation of the gradient incubator

Before use the entire apparatus was switched on and allowed to equilibrate for 24 hr. The range of temperature required within the block was then established, defined and checked for fluctuation. Initial trials had indicated that to define the gradient adequately it was only necessary to monitor the temperatures of about six tubes along the block's length.

The EEL tubes were made to allow the measurement of a passage of light through them and hence it was not necessary to remove a sample of the culture in order to measure the optical density. Growth was measured turbidometrically in a Hilger and Watts absorptiometer which accepted EEL tubes. The 500 nm. filter was found to be the most satisfactory. Each of the culture tubes was inoculated from the same exponentially growing culture to an optical density of 0·07 Hilger units. Ideally, each culture tube should have been inoculated from a culture growing at the same temperature as the tube, otherwise the inoculum would experience a temperature shock with a resultant slight lag period as demonstrated by Ng and others (1962). However, such a system is difficult to arrange and it suffers from the disadvantage that different strains might develop in the different starter cultures. The temperature shock did not appear to affect the results significantly.

Because the bacteria were originally isolated on Oxoid tryptone soya agar, the medium chosen to grow the organisms in the temperature-gradient incubator was Oxoid tryptone soya broth. However, this medium foamed excessively when air was bubbled through it and an anti-frothing agent had to be added. Two agents were tested, namely silicone anti-foaming agent (British Drug Houses Limited) at a concentration of 0·1 per cent and iso-amyl alcohol at 0·5 per cent (Marmur, 1961). Five psychrophiles were grown on the temperature gradient, one coryneform and one each of the genera *Brevibacterium*, *Pseudomonas*, *Arthrobacter* and *Cellulomonas*. The selection of the strains from within the genera was at random and the tests on which the separation of the genera was based have been described by Baker and Smith (1972). *Salmonella typhimurium* was also grown on the gradient as an example of a well-documented mesophile for comparison with the psychrophiles.

Results

A temperature range of about 30° C between the hottest and coldest tubes was generally used. A typical calibration curve of the block with the "Variac" set at 89 V and a range from 5° to 35° C is given in Fig. 3. The comparison of the effect of two anti-frothing agents on the specific growth rates of three psychrophiles is shown in Table I. The difference between the two agents was minimal so iso-amyl alcohol was used in subsequent experiments because the silicone emulsion was of uncertain composition.

The specific growth rate (k) of a batch culture is the growth rate during the exponential phase.

Hence

$$k = \frac{2 \cdot 303 \ (\log x_2 - \log x_1)}{t_2 - t_1},$$

where x_1 and x_2 are growth measurements at times t_1 and t_2, respectively. Thus, when the logarithm of optical density of a broth culture is plotted against the time at which that optical

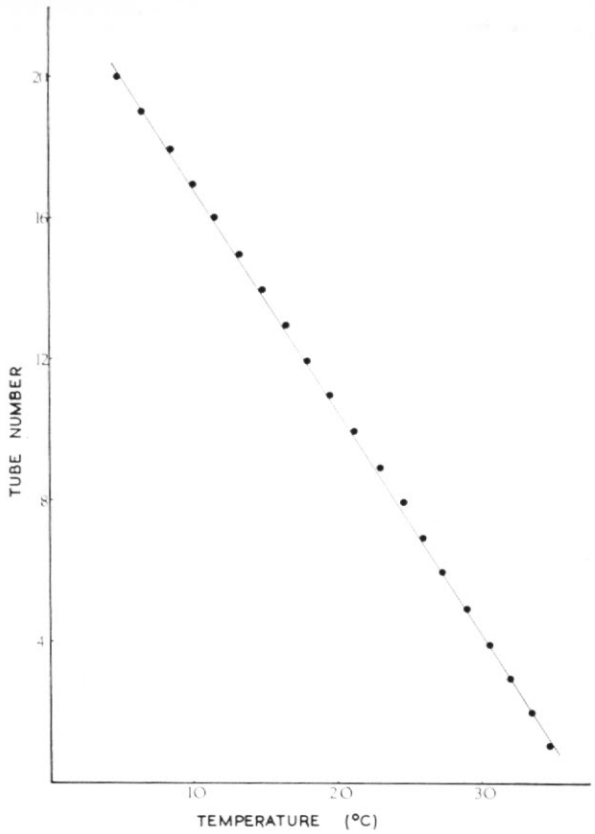

Fig. 3. A typical calibration curve of the gradient block.

TABLE I. SPECIFIC GROWTH RATES OF THREE BACTERIA IN THE PRESENCE AND ABSENCE OF TWO ANTI-FROTHING AGENTS

	Control	*B.D.H. silicone*	*Iso-amyl alcohol*
Pseudomonas sp.	0·0298	0·0233	0·0343
Achromobacter sp.	0·0750	0·0795	0·0768
Arthrobacter sp.	0·0345	0·0378	0·0359

density was measured, the resulting graph will be a straight line if the organism is growing exponentially. Fig. 4a and b are graphs of this type obtained by growing a *Cellulomonas* sp. on the temperature-gradient block. The slope of each line is proportional to its respective specific growth rate which can be calculated from the above equation. A convenient way of expressing a specific growth rate is in terms of mean generation time which is the time taken for the number of organisms in a culture to double.

$$\text{Mean generation time (hr.)} = \frac{\log_e 2}{\text{Specific growth rate}}.$$

The specific growth rates and mean generation times for *Cellulomonas* sp. growing at all the temperatures used are listed in Table II.

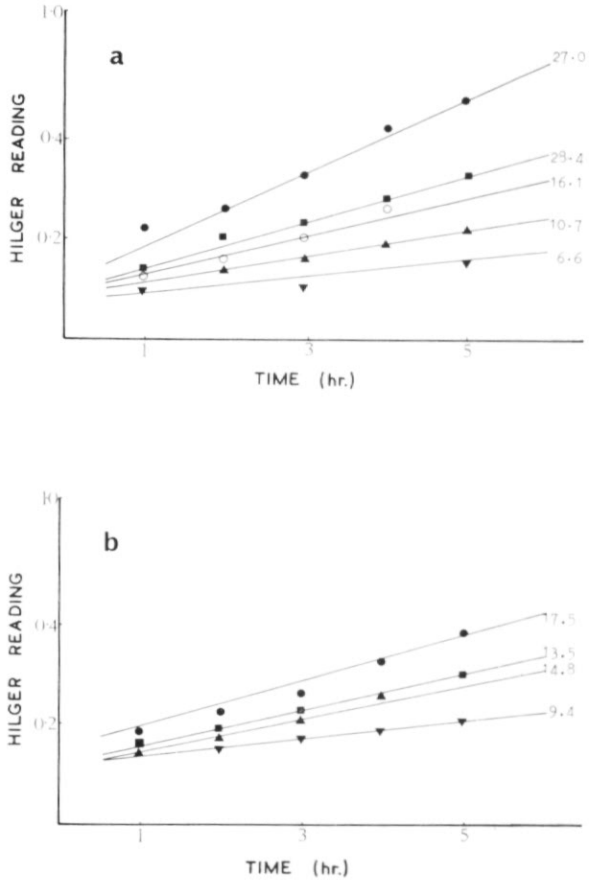

Fig. 4. a and b. Semi-logarithmic growth curves of *Cellulomonas* sp.; the numbers are the growth temperatures in °C.

Fig. 4a and b bear a striking resemblance to the graphs obtained when the influence of temperature on the rate of a first-order chemical reaction is examined. This similarity has led many microbiologists to apply the laws of chemical kinetics, particularly the Arrhenius equation, to the growth of bacterial cultures. Arrhenius (1889), following the work of van't Hoff, formulated an equation explaining the rate of a chemical reaction:

$$k^1 = Ae^{-E/RT},$$

where k^1 is the reaction rate, R is the gas constant, T is the absolute temperature, E is the activation energy and A is a constant; therefore

$$\log k^1 = \frac{E}{2{\cdot}303RT} + C;$$

then substituting growth rate for reaction rate

$$\log k \text{ (specific growth rate)} = \frac{\mu}{2{\cdot}303RT} + C.$$

Hence, if the growth of a bacterial culture can be regarded as a first-order chemical reaction, the logarithm of the specific growth rate will be inversely proportional to the absolute temperature. A graph of the logarithm of the reaction rate (or growth rate) versus the reciprocal of the

TABLE II. SPECIFIC GROWTH RATES AND DOUBLING TIMES OF *Cellulomonas* sp. OBTAINED FROM THE TEMPERATURE-GRADIENT BLOCK

Temperature (°C)	*Specific growth rate*	*Mean generation time* (hr.)
28·4	0·165	4·20
27·0	0·257	2·70
25·7	0·306	2·26
24·3	0·319	2·17
23·0	0·274	2·53
21·6	0·278	2·49
20·2	0·246	2·82
18·8	0·213	3·25
17·5	0·156	4·44
16·1	0·141	4·91
14·8	0·118	5·87
13·5	0·127	5·46
12·1	0·118	5·87
10·7	0·095	7·30
9·4	0·064	10·82
8·0	0·075	9·25
6·6	0·064	10·82

absolute temperature is called an Arrhenius plot and Fig. 5 (solid squares) is an Arrhenius plot for the *Cellulomonas* sp. derived from Table II. An Arrhenius plot for the mesophilic *Salmonella typhimurium* (solid circles) has been drawn on the same figure for comparison. The data for *S. typhimurium* were also obtained using the temperature-gradient block. The greater part of both curves is a straight line and hence the Arrhenius kinetics are applicable. It follows that the slope of the straight line is proportional to E, the activation energy, and in microbiological kinetics the term E has been replaced by μ and is called the temperature characteristic.

$$\text{Hence } \mu \text{ (temperature characteristic)} = \frac{2{\cdot}303RT_1T_2\,(\log k_2 - \log k_1)}{T_2 - T_1},$$

where k_1 and k_2 are specific growth rates at absolute temperatures T_1 and T_2, respectively.

The values for the temperature characteristics for *S. typhimurium* and *Cellulomonas* sp. are 17,400 and 15,000, respectively. No units are given because the units for the activation energy, E, are cal./mol and these are not meaningful in the context of bacterial growth. Arrhenius plots also enable the cardinal temperatures to be assessed. The point of zero slope is at the optimum temperature for growth of the organism and where the Arrhenius plot cuts the x-axis denominates the minimum and maximum temperatures for growth. Fig. 5 shows the lower part of the temperature growth range of *S. typhimurium* and the upper part of the temperature growth range of *Cellulomonas* sp. Thus it can be deduced that the minimum growth temperature of this strain of *S. typhimurium* in this particular medium is 6·5° C, and the optimum and maximum growth temperatures of the *Cellulomonas* sp. are 24·9° and 30·6° C, respectively.

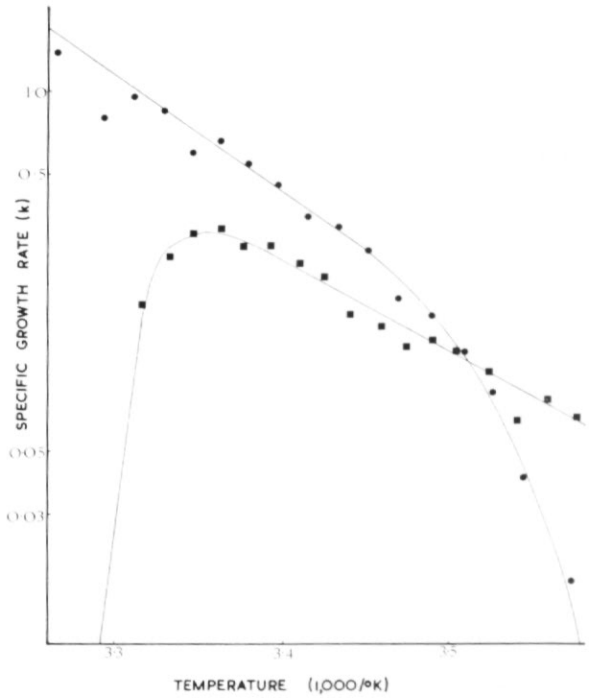

Fig. 5. Arrhenius plots of *Cellulomonas* sp. (solid squares) and *Salmonella typhimurium* (solid circles).

Corresponding graphs of growth rates and Arrhenius plots were obtained for four more psychrophiles grown in the temperature-gradient block. The temperature characteristics and cardinal temperatures for all the bacteria investigated are given in Table III.

TABLE III. CARDINAL TEMPERATURES (°C) AND μ VALUES FOR FIVE PSYCHROPHILES AND *Salmonella typhimurium*

Organism	*Optimum temperature*	*Maximum temperature*	*Temperature characteristic*
Cellulomonas sp.	24·9	30·6	15,000
Brevibacterium sp.	25·9	33·7	10,600
Pseudomonas sp.	24·7	33·1	17,800
Coryneform	28·9	31·7	10,500
Arthrobacter sp.	23·1	29·6	23,300
S. typhimurium	—	—	18,500

DISCUSSION

It has been shown that a temperature-gradient block is a convenient tool for the accurate assessment of the cardinal growth temperatures and temperature characteristics of bacteria. Determination of cardinal (particularly optimal) growth temperatures is difficult by other means (Ingraham and Stokes, 1959), as is evident from the tables of Morita (1966) in which

the cardinal temperatures of the then known psychrophilic bacteria are listed. Battley (1964) has criticized the use of this type of gradient incubator on the grounds that growth measurements can only be made at discrete temperatures and the cardinal temperature required may lie in the interval between them. This difficulty has been avoided here by evaluating the cardinal temperatures from an Arrhenius plot. Moreover, the technique is capable of providing even more accurate data, because if it was desired the temperature range of the whole block could have been concentrated over a few degrees in the vicinity of the required cardinal temperature. The alternative continuous temperature gradients are only possible using solid media which precludes the determination of growth rates.

The optimum temperatures determined (Table III) are all in the region of 25° C, a temperature they are most unlikely to meet in their native environment. This situation of microorganisms growing at sub-optimal temperatures is common and applies to most soil bacteria possibly because of the shape of the temperature *vs* growth curve, e.g. Fig. 5 (solid squares). The curve is not bilaterally symmetrical but is slewed so that the optimum temperature is much closer to the maximum than to the minimum. If the optimum growth temperature of the bacteria corresponded to the average summer soil temperature, the wide fluctuations in field temperature would mean that the maximum growth temperature was often exceeded, thereby preventing bacterial growth completely and possibly inducing death. Janota-Bassalik (1963) has also determined the growth rates of bacteria derived from peat, but her units (maximal colony count and hours required for growth of visible colonies) make a comparison difficult. However, she also gave some generation times for two psychrophiles from peat growing in a defined medium of undisclosed composition which were similar to those mean generation times reported here. Latter and Heal (1971) have also examined the growth of bacteria from Signy Island and reported that, in contrast to the present results, of 44 isolates 50 per cent grew better at 13° C than at either 1° or 25° C although the optimum temperatures were not determined. However, their method of assessing growth by visual observation of slope cultures indicates cell yield and not actual growth rates. A further difference between their study and the present work is that their bacteria were isolated from soil of a grass-covered site after storage in a deep freeze.

Harder and Veldkamp (1971) determined the specific growth rates of marine psychrophiles at different temperatures and their results are very similar to the present ones. However, these authors found a high proportion of obligate psychrophiles in the North Sea and concluded that their presence was indicative of the advantage of this type of metabolism in the marine environment. The relative scarcity of obligate psychrophiles in Antarctic peat (Baker and Smith, 1972) indicates that their conclusion cannot be extended to this particular terrestrial habitat. In a maritime Antarctic lake, however, Stanley and Rose (1967) found more than 75 per cent of the bacteria isolated were obligate psychrophiles. Possibly the more regular temperature regime of water bodies, such as the North Sea and Kroner Lake, compared with terrestrial sites results in a more favourable environment for the development of obligately psychrophilic organisms.

Interest in the temperature characteristic of psychrophiles was aroused by the work of Ingraham (1958), who stated that the temperature characteristics of three psychrophiles had values significantly lower than those of two mesophiles. He proposed that this might well be a general phenomenon of psychrophiles and hence would be an objective method of defining psychrophily. In a later publication, Ingraham (1962) stated that the value of the temperature characteristic was independent of the composition of the growth medium, an important qualification if it is to be of general use in a definition. The present work gives some support to the latter theory, because the μ value for *S. typhimurium* was 17,400 in a defined medium and 18,500 in Oxoid tryptone soya broth. The difference is not great when it is realized that a change in slope of only 2° can result in a 12 per cent change in the temperature characteristic. However, many more determinations of μ values for different organisms, both psychrophilic and mesophilic, in different media are required before the theory can gain common credence.

Ingraham's (1958) results have been challenged by both Janota-Bassalik (1963) and Hanus and Morita (1968) on the grounds that the gas constant was inexplicably omitted from the calculations of the temperature characteristics. Although this does not affect the relationship between the psychrophiles and mesophiles used by Ingraham, it has a profound effect on the

general applicability of the results. In an attempt to decide whether the μ values of psychrophiles are generally lower than those of mesophiles, some of the available data have been brought together in Table IV.

TABLE IV. A COMPARISON OF TEMPERATURE CHARACTERISTICS OF DIFFERENT BACTERIA BOTH PSYCHROPHILIC AND MESOPHILIC

Organism	*Temperature type*	*μ value*
Vibrio marinus MP1[b]	Obl. psychro.	16,200
Pseudomonas L12[e]	Obl. psychro.	11,000
Microbacterium thermosphactum[a]	Fac. psychro.	18,500
Microbacterium sp. 22[a]	Fac. psychro.	17,000
Microbacterium sp. 119[a]	Fac. psychro.	16,000
Vibrio marinus PS207[b]	Fac. psychro.	16,400
Cellulomonas sp.[f]	Fac. psychro.	15,000
Brevibacterium sp.[f]	Fac. psychro.	10,600
Pseudomonas sp.[f]	Fac. psychro.	17,800
Coryneform sp.[f]	Fac. psychro.	10,500
Arthrobacter sp.[f]	Fac. psychro.	23,300
Pseudomonas L9[e]	Fac. psychro.	11,000
Vibrio metschnekovii[b]	Mesophile	14,400
Escherichia coli[c]	Mesophile	16,000
Escherichia coli[d]	Mesophile	14,000
Salmonella typhimurium[f]	Mesophile	18,500

[a] From Brownlie (1966); [b] From Hanus and Morita (1968); [c] From Ng and others (1962); [d] From Johnson and Lewin (1946); [e] From Harder and Veldkamp (1971); [f] This paper.

The reliability of the results depends on the number of points on the linear part of the Arrhenius plot. Hanus and Morita (1968) stated that ten points are sufficient; the present work suggests that ten are a minimum and even for this number of temperatures a gradient incubating block seems essential. The use of a smaller number of points may explain the remarkable Arrhenius plot of *Pseudomonas* sp. 309 (Janota-Bassalik, 1963) which is shown with the slope decreasing with decreasing temperature. The work of Baig and Hopton (1969) is also confusing; these authors have determined the "temperature characteristic" for several psychrophiles and mesophiles but their results relate to four different 10° C intervals for each organism. As was explained earlier, the temperature characteristic for a given organism is a constant governed by the slope of a straight line. Therefore no organism can have more than one μ value as Baig and Hopton (1969) suggested and, moreover, there is no indication of which, if any, of their results is the true temperature characteristic. In order to derive any conclusions from Table IV, it must be assumed that the growth medium does not greatly affect the μ value. Given this assumption, it can be seen that there is no apparent correlation between the presence or absence of psychrophily and the value of the temperature characteristic. Hence the significance of the latter remains obscure.

ACKNOWLEDGEMENTS

I was very grateful for the opportunity to work in the department of Professor D. Lewis, F.R.S., and I wish to extend my sincere thanks to Dr. D. G. Smith, who supervised the project.

MS. received 21 January 1974

REFERENCES

ARRHENIUS, S. 1889. Über die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Saüren. *Z. phys. Chem.*, **4**, 226.

BAIG, I. A. *and* J. W. HOPTON. 1969. Psychrophilic properties and the temperature characteristic of growth of bacteria. *J. Bact.*, **100**, No. 1, 552–53.

BAKER, J. H. 1970. Quantitative study of yeasts and bacteria in a Signy Island peat. *British Antarctic Survey Bulletin*, No. 23, 51–55.

———— *and* D. G. SMITH. 1972. The genera of bacteria in an Antarctic peat. *J. appl. Bact.*, **35**, No. 4, 589–96.

BATTLEY, E. H. 1964. A thermal-gradient block for the determination of temperature relationships in micro-organisms. *Antonie van Leeuwenhoek*, **30**, No. 1, 81–96.

BROWNLIE, L. E. 1966. Effect of some environmental factors on psychrophilic microbacteria. *J. appl. Bact.*, **29**, No. 3, 447–54.

DIMMICK, R. L. 1965. Injury and growth of *Serratia marcescens* studied in a thermal gradient incubator. *Appl. Microbiol.*, **13**, No. 6, 846–50.

HANUS, F. J. *and* R. J. MORITA. 1968. Significance of the temperature characteristic of growth. *J. Bact.*, **95**, No. 2, 736–37.

HARDER, W. *and* H. VELDKAMP. 1971. Competition of marine psychrophilic bacteria at low temperatures. *Antonie van Leeuwenhoek*, **37**, No. 1, 51–63.

INGRAHAM, J. L. 1958. Growth of psychrophilic bacteria. *J. Bact.*, **76**, No. 1, 75–80.

————. 1962. Newer concepts of psychrophilic bacteria. (*In Proceedings of the low temperature microbiology symposium*. Camden, New Jersey, Campbell Soup Company, 41–56.)

————. *and* J. L. STOKES. 1959. Psychrophilic bacteria. *Bact. Rev.*, **23**, No. 2, 97–108.

JANOTA-BASSALIK, L. 1963. Growth of psychrophilic and mesophilic strains of peat bacteria. *Acta microbiol. pol.*, **12**, No. 1, 41–54.

JOHNSON, F. H. *and* J. LEWIN. 1946. The growth rate of *E. coli* in relation to temperature, quinine and coenzyme. *J. cell. comp. Physiol.*, **28**, No. 3, 47–76.

LATTER, P. M. *and* O. W. HEAL. 1971. A preliminary study of the growth of fungi and bacteria from temperate and Antarctic soils in relation to temperature. *Soil Biol. & Biochem.*, **3**, 365–79.

LONGTON, R. E. 1972. Reproduction of Antarctic mosses in the genera *Polytrichum* and *Psilopilum* with particular reference to temperature. *British Antarctic Survey Bulletin*, No. 27, 51–96.

MARMUR, J. 1961. A procedure for the isolation of deoxyribonycleic acid from micro-organisms. *J molec. Biol.*, **3**, No. 2, 208–18.

MORITA, R. Y. 1966. Marine psychrophilic bacteria. *Oceanogr. & mar. Biol.*, **4**, 105–21.

NG, H., INGRAHAM, J. L. *and* A. G. MARR. 1962. Damage and depression in *Escherichia coli* resulting from growth at low temperature. *J. Bact.*, **84**, No. 3, 331–39.

OKAMI, Y. *and* Y. SASAKI. 1967. Temperature gradient shaking machine. *Appl. Microbiol.*, **15**, No. 12, 1252–55.

PACKER, G. J. K., PRENTICE, G. A. *and* L. F. L. CLEGG. 1973. Design of a temperature gradient incubator. *J. appl Bact.*, **36**, No. 1, 173–77.

STANLEY, S. O. *and* A. H. ROSE. 1967. Bacteria and yeasts from lakes on Deception Island. (*In* SMITH, J. E., *organizer*. A discussion on the terrestrial Antarctic ecosystem. *Phil. Trans. R. Soc.*, Ser. B, **252**, No. 777, 199–207.)